



US008153397B2

(12) **United States Patent**
Smith et al.

(10) **Patent No.:** **US 8,153,397 B2**
(45) **Date of Patent:** **Apr. 10, 2012**

(54) **RECOMBINANT LIGHT CHAINS OF
BOTULINUM NEUROTOXINS AND LIGHT
CHAIN FUSION PROTEINS FOR USE IN
RESEARCH AND CLINICAL THERAPY**

C12N 1/14 (2006.01)
C12N 15/00 (2006.01)
C12N 15/63 (2006.01)
C07H 21/02 (2006.01)
C07H 21/04 (2006.01)

(75) Inventors: **Leonard A. Smith**, Clarksburg, MD
(US); **Melody Jensen**, Frederick, MD
(US)

(52) **U.S. Cl.** **435/69.1**; 435/69.7; 435/69.3;
435/70.1; 435/71.1; 435/71.2; 435/842; 435/252.3;
435/252.33; 435/254.23; 435/252.1; 435/320.1;
536/23.1; 536/23.4; 536/23.7; 536/24.1

(73) Assignee: **The United States of America as
represented by the Secretary of the
Army**, Washington, DC (US)

(58) **Field of Classification Search** None
See application file for complete search history.

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(56) **References Cited**

(21) Appl. No.: **12/387,014**

(22) Filed: **Apr. 27, 2009**

(65) **Prior Publication Data**

US 2010/0273211 A1 Oct. 28, 2010

Related U.S. Application Data

(60) Division of application No. 11/293,582, filed on Dec.
2, 2005, now abandoned, which is a division of
application No. 10/011,588, filed on Nov. 6, 2001, now
Pat. No. 7,037,680, which is a continuation-in-part of
application No. 09/910,186, filed on Jul. 20, 2001, now
Pat. No. 7,081,529, which is a continuation of
application No. 09/611,419, filed on Jul. 6, 2000, now
Pat. No. 7,214,787, which is a continuation of
application No. 08/123,975, filed on Sep. 21, 1993,
now abandoned.

(60) Provisional application No. 60/246,774, filed on Nov.
6, 2000, provisional application No. 60/311,966, filed
on Aug. 9, 2001.

(51) **Int. Cl.**

C12P 21/06 (2006.01)
C12P 21/04 (2006.01)
C12N 15/09 (2006.01)
C12N 1/12 (2006.01)
C12N 1/20 (2006.01)

U.S. PATENT DOCUMENTS

6,967,088	B1 *	11/2005	Williams et al.	435/69.1
7,037,680	B2 *	5/2006	Smith et al.	435/69.1
7,081,529	B2 *	7/2006	Smith et al.	536/23.7
7,214,787	B1 *	5/2007	Smith et al.	536/23.7
7,227,010	B2 *	6/2007	Smith	536/23.7
7,534,863	B2 *	5/2009	Steward et al.	530/350
7,632,917	B2 *	12/2009	Kincaid et al.	530/300
7,786,285	B2 *	8/2010	Smith et al.	536/23.7
7,820,411	B2 *	10/2010	Baldwin et al.	435/71.3
7,825,233	B2 *	11/2010	Steward et al.	536/23.7
2009/0017495	A1 *	1/2009	Baldwin et al.	435/69.3
2010/0196421	A1 *	8/2010	Ichtchenko et al.	424/239.1
2010/0273211	A1 *	10/2010	Smith et al.	435/69.1

OTHER PUBLICATIONS

Kadkhodayan et al, Protein Expression and Purification, Jun. 2000,
19/1:125-130.*

* cited by examiner

Primary Examiner — Nita M Minnifield

(74) *Attorney, Agent, or Firm* — Elizabeth Arwine

(57) **ABSTRACT**

The present invention relates to the construction, expression,
and purification of synthetic or recombinant light chain (LC)
botulinum neurotoxin genes from all botulinum neurotoxin
serotypes. The methods of the invention can provide 1.1 g of
the LC per liter of culture. The LC product is stable and
proteolytically active. Methods of using the products of the
invention are described.

18 Claims, 20 Drawing Sheets